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Preparation, characterization and antimicrobial activity of ferrocene-containing polymeric materials

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Abstract

Ferrocenyl(benzyl) imidazole (FcIm) and two related methyl-imidazolium salts ($FcMIm^+I^-$ and $FcMIm^+PF_6^-$) were synthesized for their incorporation into plasticized PVC by solvent casting technique. The obtained materials were investigated for their thermal stability and, compared to pure polymer, films containing ionic ferrocene derivatives in 0.5% w/w loading were found slightly more stable. The pure ferrocene compounds showed antibacterial activity against *Staphylococcus epidermis*, but not against *Escherichia coli*, with a maximum for $FcMIm^+PF_6^-$ salt. After incorporation into PVC polymer, antibacterial activity against *S. epidermis* was observed (by disk diffusion test) only for PVC/$FcMIm^+PF_6^-$ (5% w/w) film, from which a release of 14.6% of the ferrocenilimidazolium cation in aqueous medium was measured after 24 h.

1. Introduction

The antimicrobial agents, mainly grouped in disinfectants, antiseptics and antibiotics, are substances that kill or inhibit the growth of microorganism and are widely employed in the treatment of diseases and infections as well as in the sterilization of surfaces or water. Although a variety of natural and synthetic substances display effective antimicrobial activity,[1-5] the increasing occurrence of antibiotic resistance of a large number of bacteria strains together with the residual environmental toxicity of many chemical biocides has led to a growing interest both in academic and industrial fields for the design and production of new antimicrobial agents.[6] Thus, cationic surfactants, quaternary ammonium compounds, ionic liquids, peptides, natural or synthetic polymers, have been widely studied as effective alternatives to conventional biocides.[7-10]

The development of polymeric materials with antimicrobial properties has also attracted interest for their applications in health care and safety, principally for hospitalized patients. The use of such materials, obtained in most cases by incorporation of antimicrobial compounds into a suitable polymer, may be beneficial for their non-volatility, chemical stability and difficult permeation through the skin of man or animal. A variety of polymeric matrices, ranging from polyolefins to polyesters, polyurethanes and silicon polymers, together with other materials including glass, titania or nanofibers[11-14] have been investigated for the production of antimicrobial materials. In particular, polyvinyl chloride (PVC) is a versatile polymer widely used for the production of medical devices as well as enteral feeding products and novel materials with antimicrobial activity have been obtained by incorporation of metal oxides, nanoparticles and zeolites, ionic liquids and natural substances into this polymer matrix.[15-20]

In the search for molecules to be incorporated into a suitable polymer for the development of novel antibacterial materials, we focused our attention on imidazole derivatives, since their heteroaromatic unit can be considered an interesting pharmacophore[21] and its presence in a given molecular structure has been associated with different biological activities.[22-23] Furthermore, strong antimicrobial activity has been reported for imidazolium-based ionic liquids, mainly due to the alkyl chain length in the cation. Actually, imidazolium salts comprising long alkyl chains (11-16 methylene groups) easily interact with bacteria cell membrane affecting its permeability with consequent cell death.[24-25]

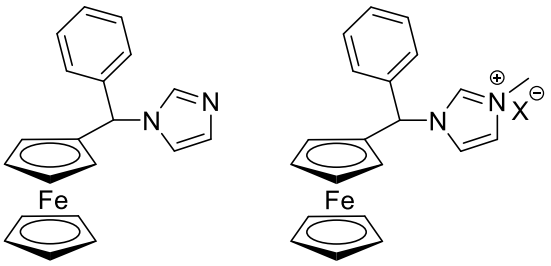
On the other hand, ferrocene derivatives have stimulated considerable interest as anticancer, antibacterial, antifungal and antiparasitic drug candidates[26] and the synthesis of "ferrocene analogues" of active drugs has led, in many instances, to enhanced activity compared to the parent molecules due to specific effects related with the redox properties of the organometallic moiety.[27-28] The combination of ferrocene and imidazole pharmacophores gave two-fold increase of cytoxicity on HT29 colorectal cancer cells for the ferrocenyl analogue of clotrimazole with respect to the parent

drug[29] and anticancer activities combined with low toxicity[30] were reported for a series of ferrocenylalkyl azoles, among which FcIm, **1**.

Although a variety of polymers with pendant ferrocenes have been prepared and their properties finely tailored for the development of stimuli-responsive smart materials,[31] ferrocene-modified thin films are less common and specifically designed for sensor applications.[32-33]

Recently, a polymer containing ferrocene triazole as pendant group has been used for stabilizing silver nanoparticles and as cross-linking agent for gelatin hydrogel to give a composite with inhibitory activities against both *Escherichia coli* and *Staphylococcus aureus.*[34] Positive activity against these microorganisms was also reported for some ferrocene-containing imidazolium type ionic liquids and the corresponding poly(ionic liquids).[35]

Prompted by the lack of data on ferrocene-containig film for biomedical application and the more simple preparation of polymer films by *solvent casting* technique with respect to the synthesis of a ferrocene-based polymer, in this study we considered ferrocene derivative FcIm, **1** and its *N*-methyl imidazolium salts (FcMIm$^+$I$^-$, **2** and FcMIm$^+$PF$_6^-$, **3**) (Scheme 1) as active compounds to be incorporated into plasticized PVC with the aim to investigate the thermal properties and the antimicrobial activity of the resultant materials and here we report the obtained results.



1, FcIm

2, X = I FcMIm$^+$I$^-$

3, X = PF$_6$ FcMim$^+$PF$_6^-$

Scheme 1. Chemical structures of the investigated ferrocenes

2. Experimental

2.1 Materials

2.1.1 General

ACS grade solvents, ferrocene and benzoylferrocene were purchased from Aldrich. PVC plasticized with tris (2-ethylhexyl) trimellitate (TOTM) having specific gravity of 1.242, hardness (shore A) of 87.5, K-value of 65.0 was supplied by Consorzio Proplast (Alessandria, ITALY). All the chemicals were high purity products and were used as received.

Column chromatography was performed on silica gel 60 (Merck, 40-63 μm) using the specified eluents.

Structural characterization of ferrocene compounds was performed by NMR and MS spectrometry. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker Avance™ 400 spectrometer at 400.13 and 100.62 MHz, respectively. Chemical shifts (δ) are given as ppm relative to the residual solvent peak and coupling constants (*J*) are in Hz. In the NMR assignment Cp and Cp' refers to substituted and unsubstituted cyclopentadienyl ring, respectively.

High resolution mass spectra (HR-MS) were obtained on a Thermo Scientific Exactive Plu Orbitrap MS (Thermo Fischer Scientific, San Jose, CA), using a heated electrospray ionization (HESI II) interface.

*2.1.2 Synthesis of ferrocenyl(benzyl) imidazole, **1** (FcIm)*

Benzoyllferrocene (1.12 g, 3.86 mmol) was dissolved in a mixture of THF/MeOH (60 ml v/v 5:1) and to this solution $NaBH_4$ (200 mg, 5.25 mmol) was added over 30 minutes, under stirring and at room temperature. The reaction was stopped by addition of H_2O (10 mL) and the solvent was then evaporated under vacuum at 40°C. The residue was suspended in EtOAc and the mixture extracted with H_2O; the organic phase was washed with saturated brine, dried over Na_2SO_4 and taken to dryness to afford 1-hydroxybenzylferrocene **4** (1.05 g, 3.58 mmol, 93% yield) as a yellow solid,[36] which was used without further purification. Compound **4** (1.05 g, 3.58 mmol), was dissolved in CH_2Cl_2 (22 mL) and to this solution acetic anhydride (1 mL) and pyridine (1 mL) were added. The mixture was left to stand at room temperature for 24 h and the solvent was then evaporated under vacuum to give 1-acetoxybenzylferrocene, **5** as an orange oil (1.16 g, 3.50 mmol, 98% yield).[36]

To a solution of **5** (1.16 g, 3.50 mmol) in CH_3CN (30 mL), imidazole (257 mg, 3.78 mmol) was added and the solution was stirred at room temperature for 24 h. The solvent was then removed under reduced pressure and the residue partitioned between EtOAc and water. The organic layer was collected, washed with saturated brine, dried over Na_2SO_4 and taken to dryness to give a residue that was purified by column chromatography on silica gel. Elution of the column with *n*-hexane:EtOAc (60:40 v/v) gave **FcIm** as yellow solid (1.02 g, 2.98 mmol, 85% yield).[37] ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.02 (br s, 1H, Cp), 4.06 (s, 5H, 5 Cp'), 4.19 (br s, 1H, Cp), 4.24 (br s, 1H, Cp), 4.28 (br s, 1H, Cp), 6.21 (s, 1H, CH), 6.84 (s, 1H, CH-Im), 7.03 (s, 1H, CH-Im), 7.19 (d, *J* = 6.8, 2H, Ar), 7.35-7.38 (m, 3H, Ar), 7.47 (s, 1H, H-2_{Im}). ^{13}C- NMR (100 MHz, $CDCl_3$) δ: 61.6 (CH), 68.2 (Cp-H), 68.3 (Cp-H), 68.4 (Cp-H), 68.7 (Cp-H), 69.0 (Cp'-H), 86.9 (Cp), 118.8 (CH-Im), 127.3 (2 × Ar-H), 128.1 (Ar-CH), 128.4 (2 × Ar-CH), 128.8 (CH-Im), 136.8 (CH-Im), 140.0 (Ar-C).

*2.1.3 Synthesis of 1-(1-phenyl-ferrocenylmethyl)-3-methylimidazolium iodide, **2** ($FcMIm^{+}I^{-}$)*

In a sealed vessel, a solution of **FcIm** (500 mg, 1.46 mmol) in CH_3I (5 mL) was refluxed at 50°C under nitrogen atmosphere until complete conversion of the substrate was reached (15 h). Following

dilution with Et_2O, **FcMIm$^+$I$^-$** separated as a yellow solid which was washed with Et_2O (3 × 10 mL) and dried at room temperature (690 mg, 1.44 mmol, 98% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 4.02 (s, 3H, N-Me), 4.08 (s, 5H, Cp'), 4.14 (s, 1H, Cp), 4.32 (s, 1H, Cp), 4.36 (s,1H, Cp), 4.54 (1H, Cp), 6.96 (s, 1H, CH), 7.06 (s, 1H, H_{Im}), 7.33 (s, 1H, H_{Im}), 7.45-7.51 (m, 5H, Ar-H), 9.41 (s, 1H, H-2_{Im}). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 37.5 (*N*-Me), 64.8 (CH), 68.3 (Cp-H), 69.6 (5 × Cp'-H), 69.8 (Cp-H), 70.1 (Cp-H), 82. 9 (Cp), 121.2 (CH-Im), 123.2 (CH-Im), 128.0 (2 × Ar-CH), 129.2 (2 × Ar-CH),129.6 (Ar-CH), 136.2 (CH-Im), 137.0 (Ar-C).

2.1.4 Synthesis of 1-(1-phenyl-ferrocenylmethyl)-3-methylimidazolium hexafluorophosphate, ***3*** *(FcMIm$^+PF_6^-$)*

To a solution of **FcMIm$^+$I$^-$** (500 mg, 1.05 mmol) in acetone (20 mL) under nitrogen atmosphere sodium hexafluorophosphate (180 mg, 1.07 mmol) was added and the reaction was carried out at room temperature for 24 h. The reaction mixture was filtered through a plug of Celite and the filtrate was concentrated under reduced pressure to give a residue, from which **FcMIm$^+$PF$_6^-$** was crystallized with *n*-hexane:CH_2Cl_2 (4:1 v/v) as a yellow solid (396 mg, 0.79 mmol, 75% yield). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.95 (s, 3H, N-Me), δ 4.07 (s, 5H, Cp'), 4.13 (s, 1H, Cp), 4.37 (s, 1H, Cp), 4.38 (s, 1H, Cp), 4.50 (s, 1H, Cp), 6.74 (s, 1H, CH), 7.04-7.28 (m, 2H, H-Im), 7.49 (m, 5H, Ar-H), 8.83 (s, 1H, H-2_{Im}). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 37.2 (*N*-Me), 65.0 (CH), 68.3 (Cp-H), 69.5 (5 × Cp'-H), 69.9 (Cp-H), 70.2 (Cp-H), 82.6 (Cp), 121.4 (CH-Im), 123.0 (CH-Im), 127.8 (2 × Ar-CH),129.3 (2 × Ar-CH),129.6 (Ar-CH), 135.8 (CH-Im), 136.8 (Ar-C).

2.1.5 Preparation of the ferrocene-containing PVC films

Before the preparation of films, the PVC pellets, FcIm and FcMim salts powders were dried at 50 °C under vacuum for 24 h. PVC-compound films were prepared by solvent casting from 10% (w/v) THF solutions (100 mL) of neat PVC (10 g) or PVC (9.95 g, 9.9 g, 9.5 g) loaded with **FcIm** or **FcMIm$^+$X$^-$** salts in 0.5 (50 mg), 1.0 (100 mg) and 5.0 % (500 mg) (w/w) concentrations with respect to PVC. The obtained solutions were kept under vigorous stirring at 40 °C for 4-5 h. Aliquots (25 mL) of the solutions were then casted on Pyrex® crystallizing dishes with flat bottom (Ø 140 mm) and the solvent was left to evaporate overnight at room temperature to give films with 100-120 µm thickness. Film samples of the same size (4 cm^2 surface) were obtained by using a tool specifically designed. To obtain reliable replicates, samples having similar weight and thickness were used for microbiological tests and FcMIm$^+$ release.

2.2 Methods

2.2.1 Calorimetric measurements

Calorimetric measurements were performed on a differential scanning calorimetry (DSC, TA Instruments Q100) equipped with a liquid sub-ambient accessory and calibrated with high purity standards (indium and cyclohexane). Nitrogen was used as purge gas. DSC measurements were carried out on a 5 mg sample in the temperature range from –90 °C to 200 °C at heating/cooling rate of 10 °C/min. The neat PVC used for all experiments was plasticized with trioctyl trimellitate (TOTM).

2.2.2 Thermogravimetric analyses (TGA)

Thermogravimetric measurements were carried out on a TA Instruments Q500. The analyses were performed under a nitrogen atmosphere at 10 °C/min heating rate, from 30 °C to 800 °C. Sample weights were approximately 4–5 mg. The weight loss percent and its derivate (DTG) were recorded as a function of temperature. The temperature of 5% weight loss was considered as the onset of degradation.

2.2.3 Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS)

Py-GC/MS analyses were performed using a Multi-Shot Pyrolyzer (EGA/PY-3030D, Frontier Labs), connected to a GC system GC-2020 (Shimadzu Corporation), coupled with a triple quadrupole mass spectrometry detector and an electronic ionization (70 eV) Mass Detector TQ8040 (Shimadzu Corporation).

For Py-GC/MS analyses an Ultra Alloy® Metal Capillary Column (Frontier Labs, stationary phase 5% di-phenyl-methylpolysiloxane, inner diameter 250 μm, film thickness 0.25 μm, length 30 m) was used. The GC oven temperature was held at 50 °C for 1 min, increased to 100 °C at 30 °C/min, then held at 100 °C for 5 min, increased from 100 °C to 300 °C at 10 °C/min and maintained at 300 °C for 10 min. The carrier gas was helium at a controlled flow of 1.78 mL/min. The split ratio was 1/50 of the total flux. Interfaces Py-GC and GC/MS were kept at 300 °C and 250 °C respectively.

A small amount of sample (about 0.1 mg) was placed, without pre-treatment, in the crucible immediately prior to the analysis and two steps of pyrolysis, at 200 °C and 305 °C, were performed in order to separate the products generated at different temperatures. Blanks were carried out before each analysis, by placing the crucible empty in the furnace and performing pyrolysis in the above conditions.

2.2.4 Microbiological assays

The antibacterial activity of pure **FcIm**, **FcMIm$^+$I$^-$** and **FcMIm$^+$PF$_6^-$** was evaluated in triplicate by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) against *Staphylococcus epidermidis* and *Escherichia coli* bacterial strains with the tube dilution broth method, according to Clinical and Laboratory Standards Institute guidelines.[38]

The strains were grown on Tryptic Soy Agar medium (TSA, Oxoid), incubated at 37°C for 24h, then collected and suspended in sterile saline solutions. Their concentration was adjusted at 10^6 cell/mL with a McFarland tube. To determine the Minimal Inhibitory Concentration (MIC) vs both *E. coli* and *S. epidermidis* strains, initial sterile saline solutions (10 mL) of the compounds **1**-**3** were prepared at a concentration of 1 mg/ml. Serial dilutions from 500 to 1.56 μg/mL were made. A bacterial suspension of *E. coli* or *S. epidermidis*, at a concentration of 10^6 cell/mL, was inoculated in each tubes, incubated at 37 °C and visually read after 24 h. The MBC was determined by subculturing in agar plate samples from the broth dilution of the lowest concentration of MIC assay in which no turbidity was shown. The subculturing broth solution that resulted in microbial death (bacteria reduction ≥99.9%) was considered as MBC of the tested antimicrobial agent.

The direct inhibition of bacteria growth was determined by disk diffusion test according to Zampino et al.[16] Round film samples of 4 cm^2 of surface (diameter ~23 mm; mean weight 80-100 mg). were used. Before microbiological analyses, samples were sterilized by irradiation with a UV lamp (wavelength 240–280 nm) for 3 min (2 cycles). Samples were placed on the surface of TSA plates, seeded with *E. coli* and *S. epidermidis*, both strains at an initial concentration of 10^6 colony forming units (CFU)/mL, and incubated at 37 °C for 48 h. The antimicrobial activity of the PVC-compound films was determined by measuring the diameter in millimeters of the inhibition zone surrounding the polymeric samples.

2.2.5 Quantification of the released $FcMIm^+$

The determination of released amount of $FcMIm^+$ was carried out by immersing round specimens (4 cm^2 surface) of PVC films loaded with 5% of compound **2** or compound **3** in tubes containing sterile water as simulant liquid and incubating at 37 °C for 24 h. Five replicate specimens were tested. The medium containing the ions released during the fixed time period was frozen and lyophilized. The obtained powder was then dissolved in CH_3CN (1.5 mL) and ferrocene (1 μg/mL) was added as internal standard. This solution was then used to quantify the amount of released $FcMIm^+$ by ESI-MS analysis. ESI Mass spectra were acquired in positive ion mode in the *m*/*z* range 120-500 at a resolving power of 25000 (full-width-at-half-maximum, at m/z 200, RFWHM). The scan rate, using automatic gain control target of 1.0 × 106 and a C-trap inject time of 100 ms, was of > 1.5 scans/sec. The following conditions were adopted: capillary temperature 300 °C, nebulizer gas (nitrogen) with a flow rate of 30 arbitrary units; auxiliary gas flow rate of 10 arbitrary units; source voltage 4 kV; capillary voltage 82.5 V; tube lens voltage 85 V. The sample (1 μL) was injected with autosampler into the mass spectrometer, using CH_3CN as solvent at a flow rate of 100 μL/min.

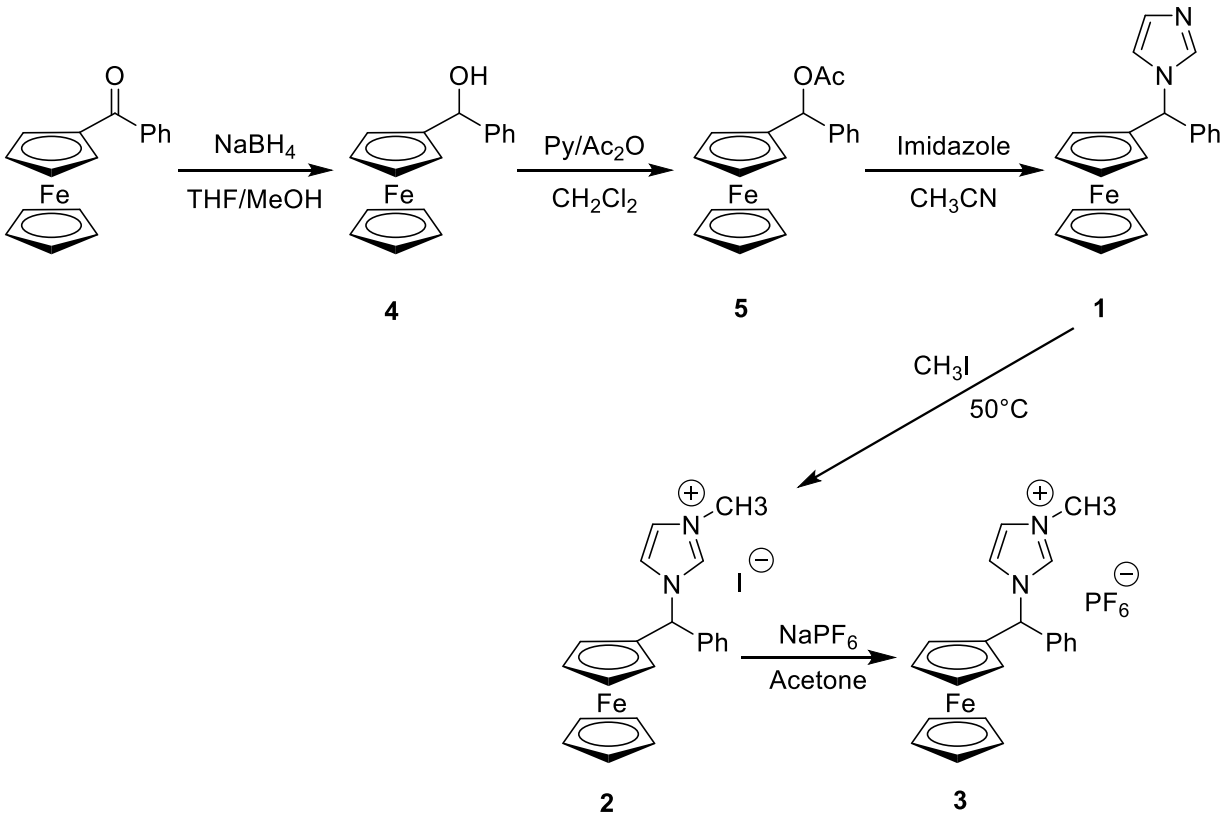
The Orbitrap MS system was tuned and calibrated in positive mode by infusion of solutions of a standard mixture of caffeine (Mr 194.1 Da), MRFA peptide (Mr 423.6 Da) and Ultramark (Mr 1621 Da). Data acquisition and analysis were performed using the Excalibur software.

For each compound a five-point calibration curve ($R^2 > 0.99$) was built using the Fc peak (*m/z* 186) as internal standard.

3. Results and discussion

3.1 Synthesis of ferrocene compounds

The synthesis of the target compounds was carried out as outlined in Scheme 2, by exploiting the reactivity of an acetoxygroup in the α-position with respect the ferrocene to be substituted by different nucleophiles in mild conditions.[39] So, starting from the known acetate **5**, the imidazole moiety was introduced in **FcIm**, which gave the corresponding iodide salt **FcMIm$^+$I$^-$** by *N*-methylation with MeI. **FcMIm$^+$PF$_6^-$** was then obtained following anion exchange of **2** in acetone, according a reported procedure.[40]



Scheme 2. Synthesis of the target ferrocenylcompounds **1**-**3**

In the ^{1}H-NMR spectrum of **FcIm** the resonances of cyclopentadienyl protons were observed as well separated signals in the typical chemical shift range of 4.0-4.3 ppm and also the protons on the

imidazole ring gave three distinct singlets, one of which (H-2_{Im}) appeared at quite low field in consequence of the deshielding exerted by the two flanking nitrogen atoms. In the spectrum of **FcMIm$^+$I$^-$** and **FcMIm$^+$PF$_6^-$** resonances of both the benzylic proton and H-2_{Im} were sensibly shifted downfield with respect to the neutral molecule **FcIm**. In more detail, the $\Delta\delta$ (= $\delta - \delta_1$) values for benzylic protons were 0.76 and 0.54 for **FcMIm$^+$I$^-$** and **FcMIm$^+$PF$_6^-$**, respectively, and also for the H-2_{Im} proton the observed $\Delta\delta$ was higher for compound **FcMIm$^+$I$^-$** compared to **FcMIm$^+$PF$_6^-$** (1.94 *vs* 1.36). These data are in agreement with the higher effective charge of I^- compared to PF_6^-, due to the different dimension of the anions ($I^- < PF_6^-$), resulting in higher electrostatic interaction experienced by the imidazolium protons in iodide salt.

Although compounds **FcMIm$^+$I$^-$** and **FcMIm$^+$PF$_6^-$** possess structural features common to ionic liquids, they are solids at room temperature, as the majority of the reported imidazolium-tagged ferrocene salts. Indeed, a variety of ferrocene-based imidazolium salts have been reported, in most cases for their use as catalysts[41-42] or receptors for anions,[43] but the derivatives possessing the features of ionic liquids are limited to few examples.[40,44]

3.1.1 Calorimetric measurements

In the DSC analysis of ferrocene derivatives (Figure S4) a T_m = 92.8 °C was observed for **FcIm** while higher T_m = 169.4 °C and 152.4 °C were measured for salts **FcMIm$^+$I$^-$** and **FcMIm$^+$PF$_6^-$**, respectively. DSC analysis of neat PVC revealed a glass transition temperature (T_g) of 61.5 °C. The thermal analysis of PVC loaded with different concentrations of **FcIm**, **FcMIm$^+$I$^-$** and **FcMIm$^+$PF$_6^-$** did not show significant variations (T_g values in the range 62-64 °C) under the adopted experimental conditions.

3.1.2 Thermogravimetric analyses (TGA)

TGA data of neat compounds and ferrocene-containing PVC materials are reported in Table I. **FcIm** and **FcMIm$^+$I$^-$** were found stable up to 224 °C and 167 °C, respectively (Table I, entries 1 and 2), whereas compound **3** displayed weight loss greater than 5% already at 50 ° C, may be for its highly hygroscopic nature. However, reliable results could be obtained by keeping compound **FcMIm$^+$PF$_6^-$** at 100 °C for 20 min before analysis (Table I, entry 3).

TGA curves (Figure 1) of **FCIm** and salts **FcMIm$^+$I$^-$** and **FcMIm$^+$PF$_6^-$** occurred in multiple steps of weight loss (one to four-five decomposition steps), the first of which is observed below 150°C and is due to the loss of water and/or organic solvents/volatiles. The weight loss steps between 200 and 300 °C are due to the thermal degradation of the compounds analyzed.

Pyrolysis data (Figure S5) obtained at 200°C and 305°C showed the formation of cyclopentadiene, methylimidazole, phenylfulvene, phenylferrocene and ferrocene together with some undefined

ferrocene adducts. At higher temperature the aromatic structures underwent crosslinking, leaving char residues of about 20% at 800°C.

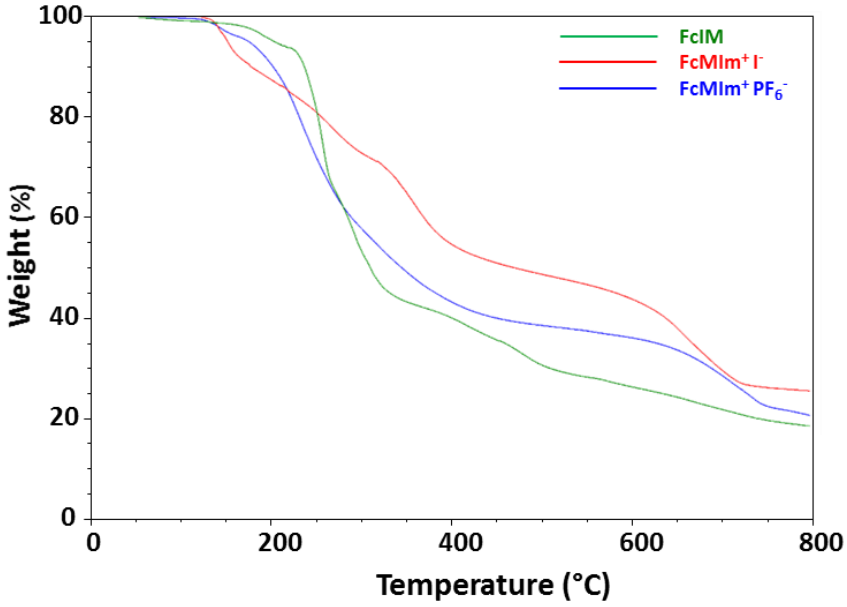


Figure 1. Thermogravimetric curves of FcIm and its salts $FcMIm^+ I^-$ and $FcMIm^+ PF_6^-$

Table I. Thermogravimetric data of neat compounds and PVC/Fc materials

Entry	Sample	$T_{\Delta m= 5\%}$ (°C)[a]	$T_{\Delta m= 50\%}$ (°C)[b]	T_{d1} (°C)[c]	T_{d2} (°C)[d]	%R[e]
1	FcIm (**1**)	224	331	--	--	21.1
2	$FcMIm^+I^-$ (**2**)	167	411	--	--	19.4
3	$FcMIm^+PF_6^-$ (**3**)[f]	196	401	--	--	20.0
4	PVC	234	308	296	476	8.9
5	PVC/ FcIm (0.5%)	229	282	264	466	13.7
6	PVC/ FcIm (1.0 %)	229	295	269	466	14.4
7	PVC/ FcIm (5.0%)	217	275	261	469	13.8
8	PVC/ $FcMIm^+I^-$ (0.5%)	248	317	307	467	12.0
9	PVC/ $FcMIm^+I^-$ (1.0%)	243	310	298	465	11.8
10	PVC/ $FcMIm^+I^-$ (5.0%)	237	295	266	455	18.9
11	PVC/ $FcMIm^+PF_6^-$ (0.5%)	256	316	297	468	9.5
12	PVC/ $FcMIm^+PF_6^-$ (1.0%)	254	313	292	468	10.4
13	PVC/ $FcMIm^+PF_6^-$ (5.0%)	238	302	288	465	12.5

[a]Onset of degradation (temperature of 5% weight loss)
[b]Onset of degradation (temperature of 50% weight loss)
[c]Temperature of maximum rate of polymer degradation (first step)
[d]Temperature of maximum rate of polymer degradation (second step)
[e]Residue (%) at 800 °C
[f]The sample was maintained at 100 °C for 20 min before analysis

Thermal degradation curves of neat PVC and PVC/**FcMIm$^+$X$^-$** compounds are shown in Figure 2. Starting from about 200 °C neat PVC experienced a marked thermal degradation mainly due to the loss of hydrochloric acid (~ 60% w/w) and a second step of degradation, related with the rearrangement of the new formed polyenic chains and their subsequent degradation, was recorded above 360 °C. As a general observation, no significant differences between neat PVC and ferrocene-containing PVCs were found at temperature below 200 °C.

The addition of **FcIm** into PVC matrix induced a general decrease of values of all the analyzed parameters even at 0.5% concentration (Table I, compare entries 5-7 with 4). Following the addition of small amounts (0.5%) of ionic ferrocene derivatives **FcMIm$^+$I$^-$** and **FcMIm$^+$PF$_6^-$** the temperature of onset of degradation (5% of weight loss) was increased by about 10 °C and 20 °C with respect to that of neat PVC, respectively, suggesting a stabilizing action of the ferrocene salts at this concentration (Table I, compare entries 8 and 11 with entry 4). By addition of higher amounts (1% or 5%) of ferrocene salts the temperature of onset of degradation gradually decreased up to values similar to that of neat PVC (Table I, compare entries 10 and 13 with entry 4).

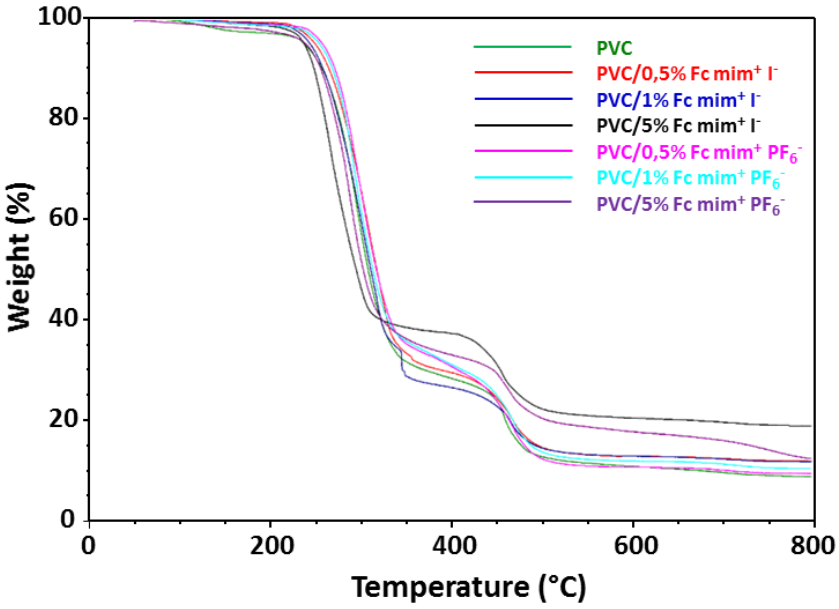


Figure 2. Thermogravimetric curves of PVC, PVC/**FcMIm$^+$I$^-$** and PVC/**FcMIm$^+$PF$_6^-$** materials

The same trend was observed for both the temperatures at which 50% of weight loss and the temperatures of the first step of degradation (T_{d1}) occur; however, the T_{d1} of PVC/ **FcMIm$^+$I$^-$** (5%) polymer was sensibly decreases by a 30 °C with respect to neat PVC (Table I, compare entry 10 with entry 4). For the second step of degradation, the variations in the T_{d2} values in comparison with the pure polymer fall within the ranges 9-21°C and 8-14°C for PVC/ **FcMIm$^+$I$^-$** and PVC/ **FcMIm$^+$PF$_6^-$** materials, respectively.

In all the samples, the thermal degradation did not decompose the whole sample and a residue, ranging from 8.9 (neat PVC) to 18.9 [PVC/ **FcMIm$^+$I$^-$** (5%)] was detected at 800 °C.

3.1.3 Microbiological assays

The synthesized ferrocene compounds **FcIm, FcMIm$^+$I$^-$** and **FcMIm$^+$PF$_6^-$** were tested for their antimicrobial activity against *S. epidermidis* and *E. coli* growth, by determining the MIC and MBC values. All the tested compounds (Table II) gave lower values of MIC and MBC for *S. epidermidis* (a Gram+ bacterium) compared to *E. coli* (a Gram– bacterium). Although **FcMIm$^+$I$^-$** and **FcMIm$^+$PF$_6^-$** share the common FcMIm$^+$ cation, **FcMIm$^+$PF$_6^-$** displayed about twofold increased inhibitory activity with respect to **FcMIm$^+$I$^-$**, indicating some role of the anion.

The antibacterial activity of imidazolium-based ionic liquids has been related with the lipophilicity of the cation, that increases with the length of the substituent alkyl chain and promotes stronger interactions with the membrane lipid layer leading to its disruption and a lethal leakage of cytoplasmic content. Although a subsidiary role is generally attributed to anions, their contribute to overall antibacterial activity, especially for short-chained imidazolium ionic liquids, has been recently evidenced and correlated with their hydrophobic and chaotropic (water-structure-breaking) properties, that positively increase the surfactant-like mode of action of these biocides.[45] The lower MIC value determined for **FcMIm$^+$PF$_6^-$** could be related with the properties of PF_6^-, which is a slightly better chaotrope[46] and more hydrophobic anion compared to I^-.

Table II. Antimicrobial assay on the two bacterial strains[a]

Compound	*S. epidermidis*		*E. coli*	
	MIC (μg/ml)	**MBC** (μg/ml)	**MIC** (μg/ml)	**MBC** (μg/ml)
FcIm	12.5	100.0	>500.0	>500.0
FcMIm$^+$I$^-$	12.5	50.0	>500.0	>500.0
FcMIm$^+$PF$_6^-$	6.2	50.0	>500.0	>500.0

[a]Experimental conditions: Bacterial strains, grown on Tryptic Soy Agar medium, were inoculated at a concentration of 10^6 cell/ml in sterile saline solutions containing the tested compounds at the suitable concentration. Incubation at 37 °C for 24 h. n=3 (three independent experiments).

The observed selectivity for *S. epidermis* is in agreement with data reported for a series of ferrocene-containing imidazolium type ionic liquids which displayed lower values of MIC for *S. aureus* (another Gram+ bacterium) compared to *E. coli* and enhanced antibacterial activity with respect to the related compounds lacking the organometallic moiety.[35] Other cationic ferrocene derivatives, structurally related with diphenylbutene, showed instead comparable activity against the two groups of microorganisms.[47]

In order to verify the direct inhibition of bacteria growth by the polymeric materials, the disk diffusion test was used and the inhibition zone surrounding the ferrocene-containing PVC films on TSA plates inoculated with *S. epidermidis* or *E. coli* was evaluated after 24 h and 48 h of incubation at 37 °C.

In comparison with neat PVC only samples containing **FcMIm$^+$PF$_6^-$** at 5% w/w concentration displayed antibacterial activity. An inhibition zone of 2.1±0.5 mm (2.6 ± 0.6 mm after 48 h) against *S. epidermidis* was induced by PVC/**FcMIm$^+$PF$_6^-$** (5% w/w) films (Figure S6) and this feature could be exploited for the use of this material for biomedical applications.

The different activity observed for **FcMIm$^+$I$^-$** and PVC/**FcMIm$^+$I$^-$** film should not be considered anomalous since it derived from different tests. Indeed, going from a solution of neat biocide (MIC test) to a polymer blend from which diffusion in agar of the biocide is required (disk diffusion test), different factors could influence the antimicrobial activity. More or less compatibility of a biocide with the polymer could affect its tendency to be released from the material, while interactions with the components of the growth media (salts, nutrients) could limit those with bacteria, so that the data on small molecules may not be confirmed. The lack of a structure-antibacterial activity correlation between imidazolium-based poly(ionic liquids) membranes and the related monomers and homopolymers has been actually reported. [48]

As a hypothesis, the different activity between the two tested PVC films could result from a better diffusion capacity in agar and/or lower interaction with the components of growth medium for **FcMIm$^+$PF$_6^-$** compared to **FcMIm$^+$I$^-$**.

3.1.4 Quantification of the released FcMIm$^+$

In order to verify if the observed antimicrobial activity of the ferrocene-containing films can be ascribed to differences in the released amounts of the active species, experiments aimed to quantification of the released FcMIm$^+$ from PVC/**FcMIm$^+$I$^-$** (5% w/w) and PVC/**FcMIm$^+$PF$_6^-$** (5% w/w) were carried out in parallel by direct infusion mass spectrometry.

Since the ESI-MS spectra of **FcMIm$^+$I$^-$** and **FcMIm$^+$PF$_6^-$** displayed a base peak at *m/z* 275, related with the fragment of molecules without the methylimidazolic moiety, and the molecular peaks were poorly detectable, the amount of released cations was determined on this peak and ferrocene, that gives an intense peak at *m/z* 186, was used as reference standard (Figure S7). A released amount of FcMIm$^+$ from the PVC/**FcMIm$^+$PF$_6^-$** (5% w/w) sample slightly higher than that from PVC/**FcMIm$^+$I$^-$** (5% w/w) was measured (14.6% *vs.* 12.5%). This finding could be related with the presence into the PVC/ **FcMIm$^+$PF$_6^-$** polymer of the PF$_6^-$ anion, whose intrinsic nature as "weakly coordinating" anion[49] and its lower effective charge compared to I$^-$ could help ionic dissociation, so promoting easier detachment of FcMIm$^+$ cations from the transient bonding with anions and their migration to the surface of polymer and consequently their release in the aqueous medium.

4. Conclusions

Neutral and ionic derivatives of ferrocenylimidazole were synthesized and incorporated into PVC polymeric films by solvent casting technique. Thermal features of the resultant materials were investigated and the presence of the ferrocene compounds did not affect the properties of the polymer. Pure ferrocene compounds **FcIm, FcMIm$^+$I$^-$** and **FcMIm$^+$PF$_6^-$** displayed moderate antibacterial activity against *S. epidermidis*, but not against *E. coli*. After incorporation into PVC, the antimicrobial activity was observed only for the hexafluorophospate salt, **FcMIm$^+$PF$_6^-$** probably in relation with its ability to be released in the aqueous medium. Although ferrocene-containing polymers are well known, the obtained results present a useful alternative in the development of ferrocene-based materials through the easy preparation of films, which is less energy demanding, cheap, and sustainable in nature.

Conflict of interest

The authors declare no conflict of interest.

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